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Successful aerobic bioremediation of groundwater contaminated with higher chlorinated phenols by indigenous degrader bacteria

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ABSTRACT

The xenobiotic priority pollutant pentachlorophenol has been used as a timber preservative in a polychlorophenol bulk synthesis product containing also tetrachlorophenol and trichlorophenol. Highly soluble chlorophenol salts have leaked into groundwater, causing severe contamination of large aquifers. Natural attenuation of higher-chlorinated phenols (HCPs: pentachlorophenol + tetrachlorophenol) at historically polluted sites has been inefficient, but a 4-year full scale *in situ* biostimulation of a chlorophenol-contaminated aquifer by circulation and re-infiltration of aerated groundwater was remarkably successful: pentachlorophenol decreased from 400 $\mu g\,L^{-1}$ to $<1\,\mu g\,L^{-1}$ and tetrachlorophenols from 4000 $\mu g\,L^{-1}$ to $<10\,\mu g\,L^{-1}$. The *pcpB* gene, the gene encoding pentachlorophenol hydroxylase - the first and rate-limiting enzyme in the only fully characterised aerobic HCP degradation pathway - was present in up to 10% of the indigenous bacteria already 4 months after the start of aeration. The novel quantitative PCR assay detected the *pcpB* gene *in situ* also in the chlorophenol plume of another historically polluted aquifer with no remediation history. Hotspot groundwater HCPs from this site were degraded efficiently during a 3-week microcosm incubation with one-time aeration but no other additives: from 5400 $\mu g\,L^{-1}$ to 1200 $\mu g\,L^{-1}$ and to 200 $\mu g\,L^{-1}$ in lightly and fully aerated microcosms, respectively, coupled with up to 2400% enrichment of the *pcpB* gene. Accumulation of lower-chlorinated metabolites was observed in neither *in situ* remediation nor microcosms, supporting the assumption that HCP removal was due to the aerobic degradation pathway where the first step limits the mineralisation rate. Our results demonstrate that bacteria capable of aerobic mineralisation of xenobiotic pentachlorophenol and tetrachlorophenol can be present at long-term polluted groundwater sites, making bioremediation by simple aeration a viable and economically attractive alternative.

1. Introduction

Pentachlorophenol (PCP) is a xenobiotic with no known natural sources (Crawford et al., 2007). Due to its high toxicity and poor biodegradability PCP is a commonly used model pollutant in environmental research. Large-scale production and use as a timber preservative from the 1930s to the 1980s, typically in a chlorophenol mixture with 2,3,4,6-tetrachlorophenol (TeCP) as the main component, resulted in persistent environmental pollution (Bryant and Schultz, 1994; Männistö et al., 2001; Rautio, 2011). In the phenolic form PCP is very poorly soluble in water, but with pK_a of 4.74 the majority of it is present as the phenolate form in near-neutral environments, rendering the sodium and potassium salts over four orders of magnitude more soluble and prone to polluting large aquifers (Olaniran and Igbinosa, 2011). The same applies to 2,3,4,6-TeCP with pK_a of 5.38. Some 30 years after banning, these

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